An Assay Developer's Tool to Enhance Diagnostic Surfaces and Improve Assay Performance

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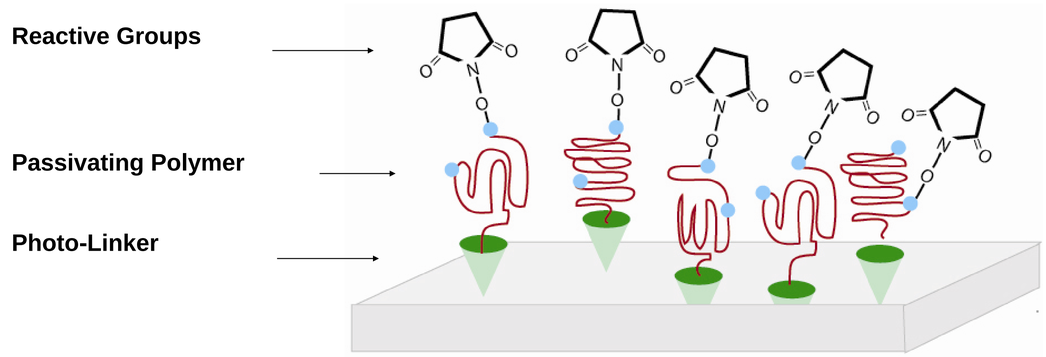
Abstract

At the core of diagnostic assays is the interface between the non-biological solid support and the sensitive biological capture analyte. Modifying the functionality of the interface surface can provide efficient attachment of the biological assay component and reduced non-specific background for improved sensitivity. Here, several interface surfaces are examined that can improve DNA, protein, and cell attachment in microarray applications. The modified interfaces examined provide superior binding and signal enhancement compared to competitor surfaces. These modified interfaces have been used in a 1x3 glass slide format. We show how these same modified interfaces may also be applied to almost any device or format to improve the nucleic acid, protein, and cell-based assay.

Introduction

Surface modification allows for the attachment of biomolecules as well as passivation of the surface to prevent non-specific binding. These UV-cured coatings are easily applied to a variety of surfaces. These include most plastics (e.g., polystryene, polyolefins, polymethylmethacrylate, silicone) as well as many inorganic substrates such as glass, silicon, and metals. The hydrophilic polymer creates a passivating layer preventing non-specific binding. Different attachment chemistries can be used to bind biomolecules such as epoxy group (TRIDIA™EP) as well as NHS esters (TRIDIA™NHS). These coatings can also be attached to different geometries allowing for the flexibility to use these unique surfaces on almost any device format. Below are three different applications highlighting the benefits these coatings provide.

TRIDIA Surface Chemistry



Analysis of Troponin on a Protein Microarray

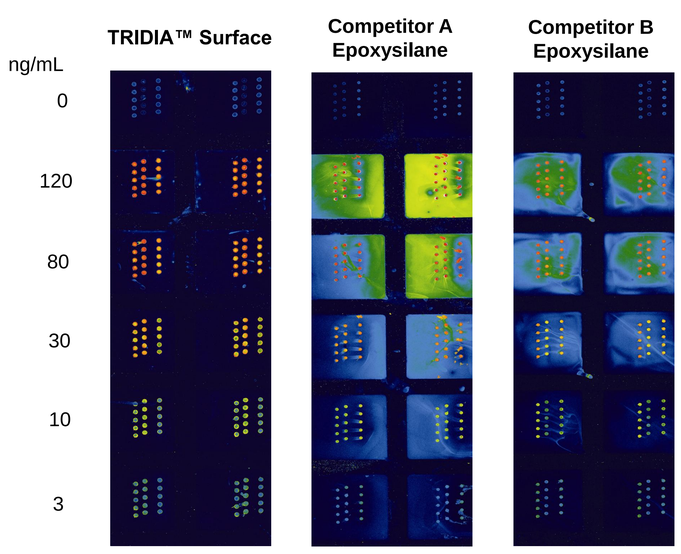


Figure 1

Troponin I sandwich immunoassay. Capture antibody – mouse anti-troponin I (AbCam), Primary detection antibody -rabbit anti-troponin I antibody, Secondary detection antibody -goat anti-rabbit-Cy3. Scanned on Axon 4200 AL scanner in the 632 nm channel.

The images above show the ability of the *TRIDIA* surface to block non-specific binding of assay components compared to commercially available epoxysilane surfaces. The epoxysilane slides show non-specific binding at the higher troponin levels. The *TRIDIA* slide displays more distinct spots with consistently low background across the slide.

DNA Oligonucleotide Binding to Microarray Slide Surfaces

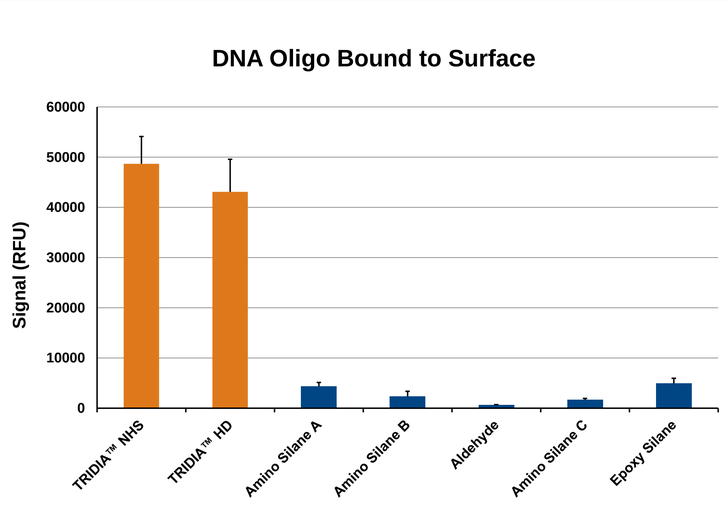


Figure 2

Biotinylated oligonucleotide was attached either through a 5'amine group or hydroxyl group depending on the surface tested. Streptavidin-Cy5 was used for detection and quantification of oligo bound to surface.

The *TRIDIA* NHS and *TRIDIA* HD surfaces have superior DNA binding compared to other common microarray surfaces.

Attachment of HUVEC in a Cell Array

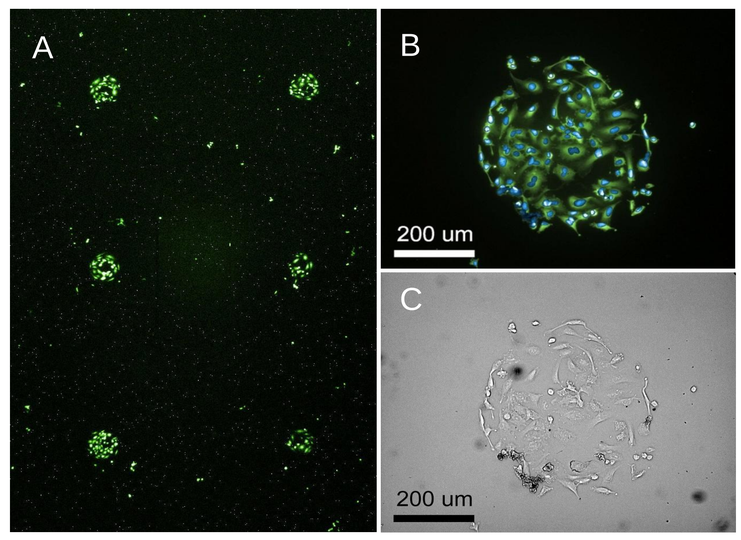


Figure 3

Cell Attachment. RGD peptide was attached to *TRIDIA* slides and the surface sterilized by UV light. HUVEC cells stained with Calcein AM (Molecular Probes) were grown for 3 days on the surface. Cells were fixed with paraformaldehyde and imaged.

The images above demonstrate that the *TRIDIA* surface binds a factor that promotes cell adhesion and eliminates non-specific binding of cells to the remainder of the surface. Image A is a fluorescent image of 6 RGD spots on a *TRIDIA* slide. Image B is a magnified spot on the array more clearly showing the attachment of the cells only in the spot where the RGD was printed. Image C is a phase contrast image of the same spot.

Summary

SurModics offers custom coatings for Molecular Diagnostic and Immunoassay applications, including nucleic acid, protein, and cell-based assays. SurModics' TRIDIA™ surface coatings bind molecules to a variety of surfaces and geometries and may be customized for selectivity using passivating polymers and reactive groups. The passivating polymer prevents non-specific binding of unwanted biomaterial in samples, while the specific reactive groups increase signal by providing higher loading densities of the capture bioanalyte.

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